Development 121, 1263-1272 (1995)
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wingless induces transdetermination in developing *Drosophila* imaginal discs*

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*Dedicated to the memory of Dr Ernst Hadorn

SUMMARY

***Drosophila* imaginal discs, the precursors of the adult fly appendages, have been the subject of intensive developmental studies, particularly on cell determination. Cultured disc fragments are recognized not only for the ability to maintain their determined state through extra cell divisions but also for the ability to transdetermine, or switch to the determined state of a different disc. An understanding of transdetermination at a molecular level will provide further insight into the requirements for maintaining cell determination. We find that ectopic expression of the *Drosophila* gene *wingless* induces transdetermination of foreleg imaginal disc cells to wing cells. This transdetermination occurs in foreleg discs of developing larvae without disc fragmentation. The in situ-transdetermining cells localize to the dorsal region of the foreleg disc. This *wingless*-induced transdetermination event is remarkably similar to the leg-to-wing switch that occurs after leg disc culture. Thus we have identified a new approach to a molecular dissection of transdetermination.**

Key words: transdetermination, *wingless*, leg imaginal disc, *Drosophila*

INTRODUCTION

Once determined, a cell is committed to a particular differentiative pathway. However, differentiation may be immediate or delayed. A fundamental question in developmental biology is: how do cells maintain a stably determined state? *Drosophila* imaginal discs have provided a unique system for the study of cell determination because imaginal cells must maintain their disc-specific determination from the time they are established during embryogenesis, through a prolonged period of cell divisions, until they differentiate at metamorphosis. Imaginal disc transplantation experiments have shown that disc cell determination is stably cell-heritable not only during larval development but also following extensive periods of proliferation during in vivo culture (Hadorn, 1965). However, in some cases a few cells of cultured disc fragments alter their state of determination to another disc identity. For example, when cultured leg disc fragments are allowed to differentiate, some of the leg cells may differentiate into wing structures. This switch in cell determination is called transdetermination (Hadorn, 1965).

Transdetermination is of general interest because similar morphological transformations can occur in other organisms. Homeotic regeneration occurs in hemimetabolous insects when, for example, a broken antenna regenerates as a leg (Bateson, 1894; Chan, 1993). In certain species of frogs, retinoids can induce amputated tadpole tails to regenerate as hind limbs (Mohanty-Hejmadi et al., 1992; Maden, 1993). In these phenomena, as in imaginal disc cell transdetermination, the maintenance of a determined state is altered to another developmental program. An analysis of the switches involved in imaginal disc cell transdetermination will reveal how such mistakes in propagating specific determined states occur.

Imaginal disc cell transdetermination is not a random event. Different disc types transdetermine with characteristic frequencies to specific structures and always produce the same initial transdetermined pattern (reviewed by Hadorn, 1978). Transdetermination can be initiated by only a few cells, and many discs have localized regions of cells with a greater potential to transdetermine (reviewed by Hadorn, 1978). Cell proliferation is required but is not sufficient for transdetermination (Tobler, 1966; Wildermuth, 1968; Shearn et al., 1984). Intriguingly, many transdetermination events resemble the phenotypes of homeotic mutants. For example, transdetermination from antenna to leg mimics the *Antennapedia* mutation, and ectopic expression of *Antennapedia* can induce the antenna-to-leg transformation (Schneuwly et al., 1987). Various mutagens have been reported to induce the antenna-to-leg transformation (reviewed by Postlethwait and Schneiderman, 1973). However, induction of random cell death, which leads to disc cell proliferation and pattern duplications (Russell et al., 1977), has failed to elicit transdetermination (M. Russell, personal communication).

Besides the homeotic genes, the segment polarity genes are instrumental in specifying disc cell identity (see reviews by Wilkins and Gubb, 1991; Cohen, 1993). The segment polarity gene *wingless* (*wg*) (Nüsslein-Volhard and Wieschaus, 1980) encodes a secreted intercellular signaling molecule (DiNardo et al., 1988; Martinez Arias et al., 1988; van den Heuvel et al., 1989; González et al., 1991) that is a member of the *Wnt*

gene family (Rijsewijk et al., 1987; reviewed by Nusse and Varmus, 1992). *wg* is required not only for establishing imaginal disc primordia in the *Drosophila* embryo (Simcox et al., 1989) but also for proper imaginal patterning (Sharma and Chopra, 1976; Morata and Lawrence, 1977; Baker, 1988a). In leg discs, *wg* is expressed in an anterior-ventral sector throughout development (Baker, 1988b; Couso et al., 1993; Diaz-Benjumea and Cohen, 1994). Reduction-of-function *wg* mutations cause a loss of ventral leg structures and a mirror-image duplication of dorsal leg structures (Baker, 1988b; Peifer et al., 1991; Held et al., 1994). Ectopic expression of *wg* in leg discs can reorganize the leg pattern in a non-autonomous manner, producing mirror-symmetric ventral leg pattern duplications and supernumerary appendages (Struhl and Basler, 1993). These events require extra cell proliferation that may be induced by *wg* (Skaer and Martinez Arias, 1992; Kaphingst and Kunes, 1994). Because *wg* activity correlates with both cell proliferation and disc pattern regulation and also plays a role in homeotic gene regulation (Thüringer and Bienz, 1993; Thüringer et al., 1993), *wg* is a good candidate to be involved in transdetermination.

We report here that ectopic expression of *wg* induces transdetermination of foreleg disc cells to wing cells. This transdetermination event occurs in developing discs, yet mimics the properties of disc fragmentation experiments. We find that *wg* acts to alter the fates of only dorsal leg disc cells to ventral wing cells. Thus ectopic expression of a gene normally used for imaginal disc development can cause localized transdetermination.

MATERIALS AND METHODS

Fly stocks and the '*flp*-out' technique

Fly stocks carrying the *hsp70-flp*, *Act5C>y⁺>wg* (two independent insertion lines) and *Act5C>y⁺>sc* (for a control) transgenes were provided by Gary Struhl (Struhl and Basler, 1993). The *hsp70-flp* transgene is a fusion of the heat shock-inducible *hsp70* promoter with the site-specific *flp* recombinase gene. The *Act5C>y⁺>wg* transgene has a cell marker gene *yellow* (y^+) flanked by *flp* recombination target (FRT) sites (>), a constitutive actin promoter (*Act5C*) upstream, and the wild-type *wg* coding sequence downstream. The *Act5C>y⁺>sc* transgene is similarly constructed with the wild-type *scute* (*sc*) coding sequence downstream. Following the convention of Struhl and Basler (1993), only the *y* gene is labeled with a '⁺' to distinguish the wild-type gene from the *y* mutation. We employed the '*flp*-out' method of Struhl and Basler (1993) to generate clones of imaginal disc cells that ectopically express *wg* (or *sc*). Briefly, fly stocks carrying the *hsp70-flp* and *Act5C>y⁺>wg* (or *Act5C>y⁺>sc*) transgenes are crossed to produce larvae that carry one copy of each transgene in a *y* background. Heat shocking these larvae activates the *flp* recombinase, which can then act at the FRT sites to '*flp*-out' the y^+ gene. *wg* (or *sc*) expression is then heritably activated by the *Act5C* promoter in cells that are genetically and phenotypically *y*. For the experiments described here, we made 1-hour collections of eggs (after 1-hour pre-collections) from the above crosses at 25°C on standard media. When raised in uncrowded conditions, such collections yield well-synchronized cultures of larvae. Experimental larvae were heat-shocked at 60 hours after egg laying (mid-2nd instar stage) for 20 minutes at 33-34°C. Following the heat shock, larvae continued development at 25ºC until the first larvae started wandering (cultures of larvae that are induced to ectopically express *wg* lose their synchrony after a heat shock), at which time the cultures were switched to 18°C to allow more wandering larvae to be collected for disc dissections. These larvae either were then used for immunocytochemistry or were allowed to differentiate and were used for cuticle analysis.

Immunocytochemistry

Discs were dissected from both control (non-heat shocked) and experimental (heat shocked) wandering stage male larvae in Ringer's solution and were accumulated in Ringer's in microtiter wells on ice. Discs were fixed in 2% paraformaldehyde in Brower buffer (Brower, 1987) for 2 hours at 4ºC. After fixation, discs were rinsed in PBNT (0.5 M NaCl, 0.01 M $NaPO_4$, 1% BSA, 0.1% Triton X-100), then blocked in PBNT with 10% normal goat serum for 30 minutes at 4ºC. All subsequent antibody dilutions and washes were in PBNT with 1% normal goat serum at 4ºC. Polyclonal serum against Vestigial, provided by Jim Williams and Sean Carroll, was used at 1:200 dilution with an overnight incubation. A goat anti-rabbit Texas Red-conjugated secondary antibody (Jackson ImmunoResearch) was used at 1:200 dilution (2-hour incubation) to generate a fluorescent signal. After a final rinse in PBS, discs were mounted in 90% glycerol with 1% n-propyl gallate in PBS and examined using a Bio-Rad MRC 600 confocal microscope.

Cuticle analysis

Although about 50% of the animals develop to eclosion following the heat shock protocol to induce ectopic *wg* clones, many die as pharate (uneclosed but differentiated) adults. All eclosed and pharate adults from a heat shock were collected and stored in 70% ethanol. Male forelegs were dissected from these animals in water and mounted in Faure's water mounting medium (Lee and Gerhart, 1973). Cuticle was viewed using a Wild compound microscope with blue-filtered transmitted light. Foreleg cuticle morphology was analyzed based on the chaetotaxy of Hannah-Alava (1958) and the foreleg disc fate map (Schubiger, 1968).

As a control for *y* clone distribution, clones of ectopic *sc* expression (Struhl and Basler, 1993) were induced in *hsp70-flp; Act5C>y⁺>sc* larvae using the identical heat shock protocol for generating ectopic *wg* clones. We observe no significant lethality after heat-shocking these animals. Eclosed flies (no pharate adults were found) were collected and analyzed as described above.

RESULTS

Ectopic *wingless* expression induces *vestigial* expression in foreleg imaginal discs

To determine whether *wg* is capable of eliciting transdetermination, we have induced clones of imaginal disc cells that ectopically express *wg* using the heat-inducible '*flp*-out' technique of Struhl and Basler (1993). We have focused our analysis on the male foreleg disc because cells of certain male foreleg disc fragments transdetermine readily to wing cells when allowed just a few extra cell divisions after transplantation (Schubiger, 1971). As a marker for transdetermination from leg to wing, we have used the *vestigial* (*vg*) gene product, which is normally expressed in wing discs but not in leg discs (Fig. 1A,B) (Williams et al., 1991, 1993). In mid second instar wing discs, *vg* has a strong, ubiquitous expression pattern, which is refined in third instar wing discs such that strongest expression of *vg* occurs in the presumptive wing blade and in a portion of the presumptive wing hinge regions (Fig. 1A) (Williams et al., 1993). *vg* is required for wing development (Stanley, 1931; Williams and Bell, 1988) and is dependent on *wg* for proper expression in the wing disc (Williams et al., 1993).

We find ectopic Vg staining in 6% (10/160 discs) of male

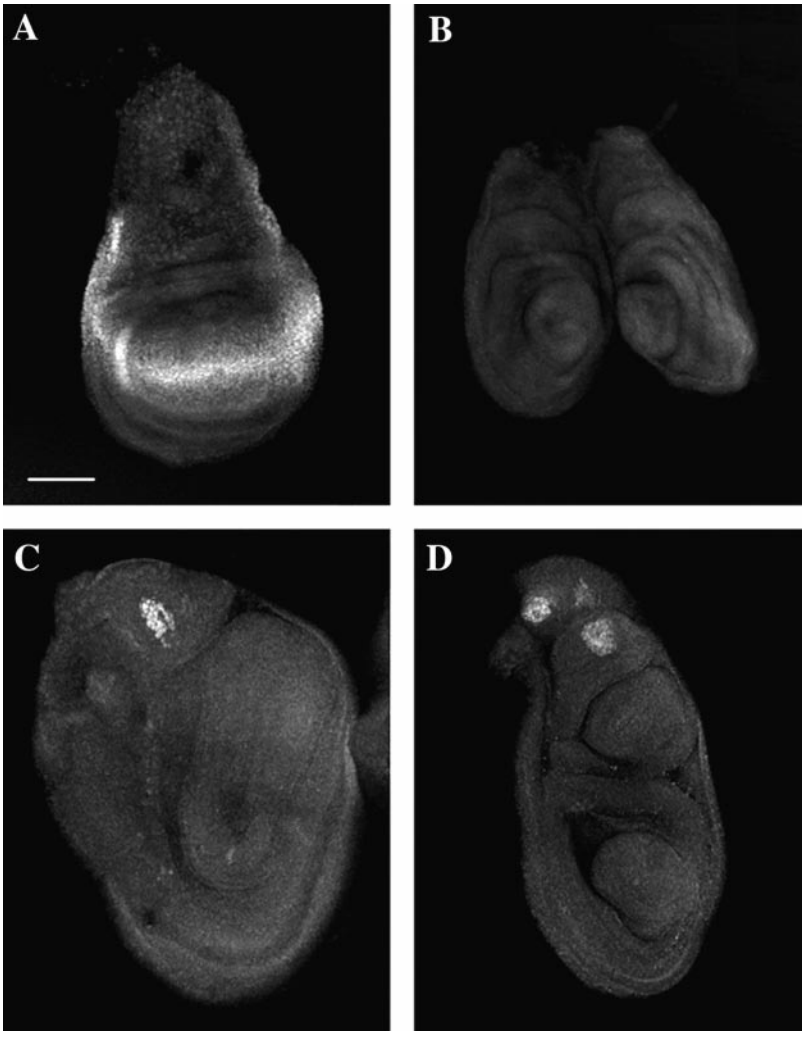

Fig. 1. Ectopic *wg* expression induces *vg* expression in foreleg discs. (A,B) Control (non-heat shocked) discs. (C,D) Experimental (heat-shocked) leg discs that have been induced to ectopically express *wg*. All discs are shown dorsal side up. A, C and D are shown anterior to the left. (A) Vg immunostaining in control late third larval instar wing disc. At this stage, Vg is expressed strongly in the presumptive wing blade and in a portion of the presumptive wing hinge regions of the wing disc (Williams et al., 1991, 1993). Vg is also expressed in the haltere disc throughout development but is not normally expressed in leg discs at any stage of development (Williams et al., 1991). (B) Pair of control late third larval instar male foreleg discs showing no Vg immunostaining. (C,D) Ectopic Vg immunostaining in experimental male foreleg discs appears in round clusters of cells that are always located in proximal-dorsal regions of the disc. These discs are overgrown (compare Fig. 1C,D with B) and can have an abnormal U-shaped morphology (compare Fig. 1C with B) and duplicated distal primordia (compare Fig. 1C,D with B). All discs are shown at the same magnification. Scale bar, 50 μm.

foreleg discs that have been induced to ectopically express *wg* (Fig. 1C,D). This ectopic Vg staining always occurs in proximal-dorsal patches of cells, suggesting that ectopic *wg* expression induces transdetermination of these specific cells from leg to wing. Moreover, leg discs with Vg staining are overgrown (compare Fig. 1C,D with B) and often have an abnormal U-shaped morphology (compare Fig. 1C with B) as well as duplicated distal primordia (compare Fig. 1C,D with B), indicating that disc cell proliferation and pattern regulation accompany transdetermination.

Ectopic *wg* expression induces transdetermined wing structures in foreleg cuticle

To test if indeed *wg* can cause the formation of transdetermined wing structures, we have analyzed differentiated male foreleg cuticle from flies in which clones of ectopic *wg* expression have been induced. Wing structures contiguous with leg cuticle (Fig. 2B-D) occur with a frequency of 4% (9/202 legs), consistent with the frequency of ectopic Vg immunostaining. Out of this group of legs from both eclosed and pharate adults, transdetermination is found only in legs from pharate adults (8% of pharate adult legs, 9/102 legs). Transdetermination is also observed after inducing ectopic *wg* expression in a second line of flies with an independent insertion of the *Act5C>y*$^{+}$*>wg* cassette (16% of pharate adult legs, 7/43 legs). In all, 31 transdetermined legs from these and several other heat shock experiments have been analyzed. In these legs, only relatively small areas of wing hinge structures can be identified. Wing hinge hairs are observed in all transdetermined legs (Fig. 2B,D). Other specific wing hinge structures, such as the yellow club and the pleural wing process (Fig. 2C), occur with lower frequencies (see Fig. 3A legend). All identified transdetermined wing structures fate map to the presumptive ventral hinge region of the wing disc (Fig. 3A; see Bryant, 1975 for a description of wing morphology and a more detailed wing disc fate map). These wing structures are only observed adjacent to the dorsal region of leg segments (Fig. 2B,D), correlating well with the dorsal location of ectopic Vg staining in leg discs. The wing structures are usually associated with proximal leg segments, however they can also appear in tarsal segments (data not shown).

Several observations suggest that *wg* induces this transdetermination event. Transdetermination is never observed in non-heat shocked *Act5C>y*$^{+}$*>wg* controls (*n*=113 legs) or in controls with heat shock-induced *sc*-expressing clones (*n*=128 legs). Ectopic *sc* expression causes the formation of extra leg bristles and sensilla but does not have any further effect on leg patterning (data not shown; Struhl and Basler, 1993). The *sc*

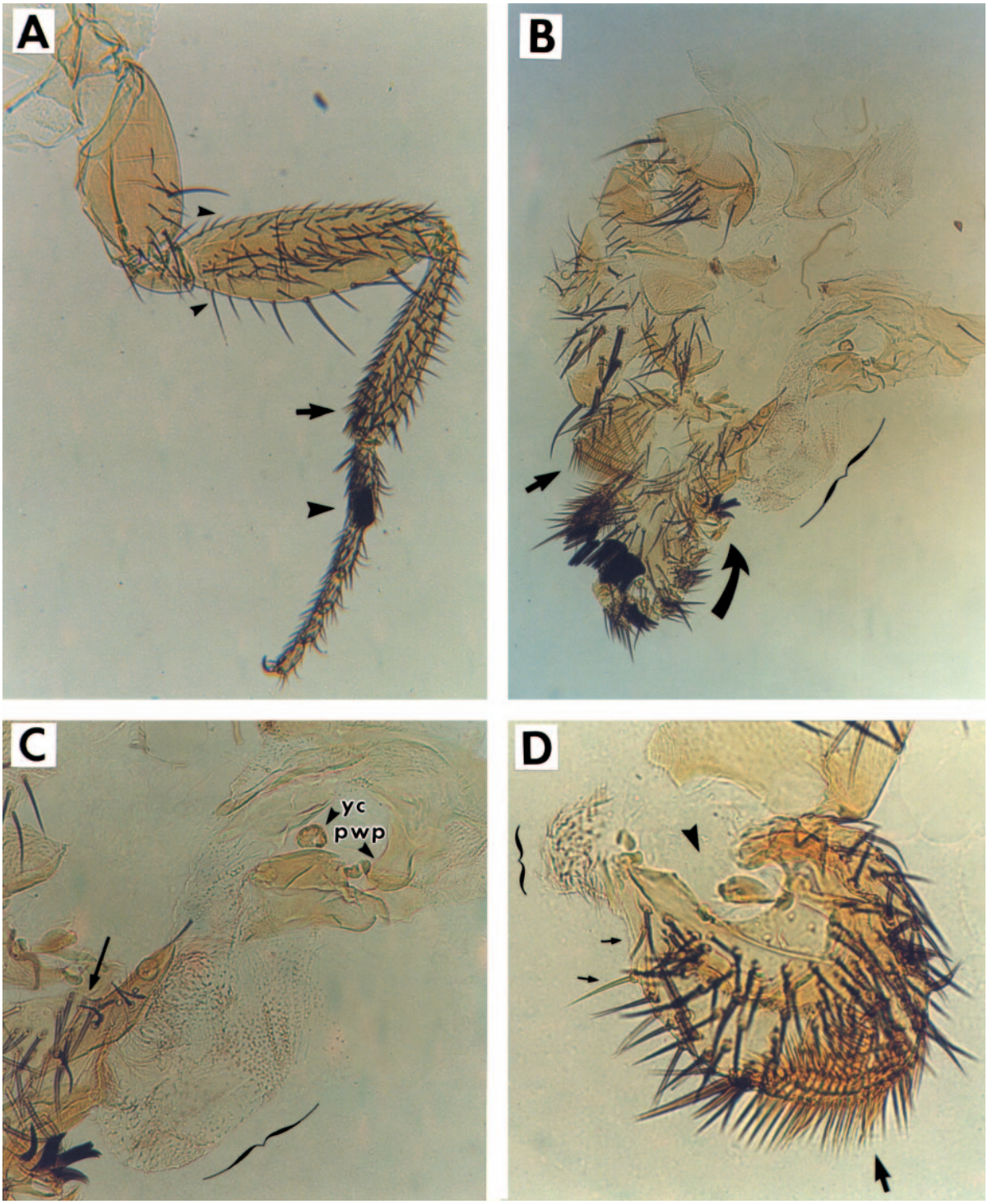

Fig. 2. Transdetermination induced by ectopic *wg* expression can be observed in differentiated foreleg cuticle. (A) Control male foreleg showing the longitudinal bristle rows (small arrowheads) of the femur, the yellow transverse rows (arrow) of the tibia, and the sex comb (large arrowhead) of the first tarsal segment. (B) A male foreleg that was dissected from a pharate adult with *y*, *wg*-expressing clones. A patch of wing hinge hairs (bracket) is found adjacent to the dorsal sides of the femur and distal leg segments. Dorsal leg structures are lost along the entire proximal-distal axis of this leg. Note that the tibia consists almost solely of ventral transverse bristle rows (arrow) and that the distal tarsal segments have not properly everted and curl dorsally (follow curved arrow; tip of curved arrow points to a multiplied claw). (C) Higher magnification view of B. A patch of wing hinge hairs (bracket) is contiguous with leg cuticle containing *y*, *wg*-expressing leg bristles (arrow). Also present are the yellow club (yc) and the pleural wing process (pwp). (D) High magnification view of a tibia segment from a male foreleg with *y*, *wg*-expressing clones and transdetermination. The circumference of the tibia segment is open on the dorsal side (arrowhead), directly opposite the ventral transverse bristle rows (large arrow). Wing hinge hairs are adjacent to the dorsal opening (bracket). Yellow leg bristles (small arrows) are found adjacent to the transdetermined wing. Magnification is ×65 in A; ×100 in B; ×200 in C; and ×250 in D.

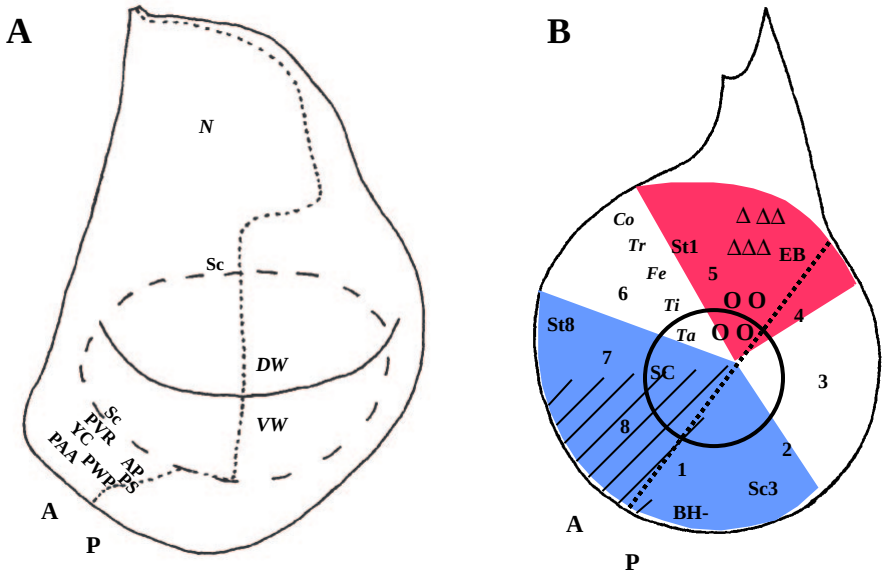

Fig. 3. Identification of cuticle structures observed in transdetermined legs. (A) Wing structures observed in 31 transdetermined male forelegs are labeled on a fate map of a third larval instar wing disc (Bryant, 1975). We identify the following wing hinge structures in legs with ectopic *wg* expression (frequencies are given in parentheses): Sc=sensilla campaniformia (11/31), YC=yellow club (11/31), PVR=proximal ventral radius (10/31), PWP=pleural wing process (7/31), PS=pleural sclerite (4/31), AP=axillary pouch (2/31), PAA=prealar apophysis (2/31). These structures cluster at the presumptive ventral wing hinge region. Groups of sensilla campaniformia also map to the presumptive dorsal wing hinge region; we cannot distinguish between ventral and dorsal sensilla. Also labeled, for reference, are the presumptive regions for the notum (*N*), the dorsal wing surface (*DW*), and the ventral wing surface (*VW*). (B) Foreleg pattern elements that are frequently duplicated and multiplied (blue shading) or lost (red shading) in 28 transdetermined male forelegs are plotted on a fate map of a third larval instar male foreleg disc (Schubiger, 1968). Data for this figure is presented in Table 1. Representative leg pattern elements are included to show their relative positions: BH$^-$ bristle of the coxa, Sc3 sensilla group of the trochanter, sex comb (SC) of the first tarsal segment, St8 sensilla group of the coxa, St1 sensillum of the trochanter, and the edge bristle (EB) of the trochanter. Also plotted are representative positions where supernumerary legs bifurcate from the endogenous legs (circles) and representative positions where transdetermined wing structures are found (triangles). Primordia for the individual leg segments, essentially a set of concentric circles, are labeled along a radius of the leg disc fate map. From proximal to distal: *Co*=coxa, *Tr*=trochanter, *Fe*=femur, *Ti*=tibia, *Ta*=tarsi. Hatched region marks the normal domain of *wg* expression (Baker, 1988b; Couso et al., 1993; Struhl and Basler, 1993), although its exact position relative to markers on the fate map is unknown. Numbers 1-8 indicate fate map positions of the longitudinal bristle rows of the femur (Hannah-Alava, 1958; Steiner, 1976), given for reference for Table 2. Both fate maps are shown dorsal side up, anterior to the left. Dotted lines indicate positions of anterior-posterior (A/P) compartment boundaries (Brower et al., 1981; Steiner, 1976).

control shows that neither the heat shock protocol, the '*flp*-out' technique, nor the generation of additional bristles cause transdetermination. More significantly, *y*, *wg*-expressing clones are always observed in dorsally positioned leg bristles adjacent to the transdetermined wing cuticle (Fig. 2C,D). Because we cannot resolve *y* clones in wing hinge hairs, we cannot determine whether or not ectopic *wg*-expressing clones directly contribute to the transdetermined structures. However, *wg*, which encodes a secreted protein, may act non-autonomously in these dorsal leg clones to induce transdetermination.

Gain of ventral leg structures and loss of dorsal leg structures always accompany transdetermination to wing

Two foreleg pattern deviations always accompany this transdetermination event: gain of ventral leg structures and loss of dorsal leg structures (Table 1). For example, all transdetermined legs that could be scored for specific leg pattern elements have extra sex combs and are missing the dorsal St4 group of sensilla (Table 1). We identify two types of gain of ventral leg structures. First, ventral leg sensory organs and bristles are locally duplicated or multiplied. Fig. 4A illustrates these events. In this leg, the ventral BH$^-$ bristle of the coxa is multiplied from 1 bristle to 5. Also, the trochanter's ventral St5 group of sensilla has duplicated to 2 groups, and one of the groups has multiplied from 5 to 17 sensilla. Second, along with the localized increase of ventral leg structures, 58% (18/31) of transdetermined legs have distal leg bifurcations. These outgrowths emerge from the dorsal side of distal leg segments yet consist of ventrolateral leg structures, such as sex comb bristles, thus making mirror-symmetric supernumerary appendages (Fig. 4B). Similar bifurcations have been previously observed in legs with ectopic *wg*-expressing clones (Struhl and Basler, 1993; Diaz-Benjumea and Cohen, 1994). Consistent with these previous studies, *y*, *wg*-expressing clones contribute to these outgrowths (Fig. 4B).

In contrast to the gain of ventral structures, dorsal leg structures are lost in legs with transdetermination. Individual leg segments are often U-shaped, with the circumference open dorsally (Figs 2D, 4A). Transdetermined legs that do not have distal outgrowths have dorsal loss in all leg segments, such that the tarsal segments curl dorsally (Fig. 2B). Transdetermined legs that have distal outgrowths have dorsal loss only proximal to the bifurcation (Fig. 4B), such that the distal segments of the endogenous leg are circumferentially complete. Interestingly, the claws, which are believed to have a dorsal position on the foreleg disc fate map (Schubiger, 1968; Held et al., 1994), are never lost but are often duplicated or multiplied (Table 1; Fig. 2B). Fig. 3B illustrates where the duplications, multiplications, bifurcations and losses that accompany transdetermination are positioned on a third instar male foreleg disc fate map.

These pattern regulation events that accompany transdetermination in adult legs are also apparent during disc development. Both leg discs and leg segments appear U-shaped (Figs 1C, 2D, 4A), apparently because of the loss of dorsal struc-

Table 1. Gain of ventral leg structures and loss of dorsal leg structures occur in transdetermined legs

	Leg pattern elements (n=28 legs)																
	Coxa				Trochanter								Femur		Tibia	Tarsi	
	V	→		D	V	→		→		→		D	V	D	D	V	D
	BH$^-$	St8	St3	St4	St5	Sc3	GSt1	GSt2	Sc$^+$5	St1	EB	Sc$^-$8	Sc11	Sc1	PA	SC	Claw
No. Dup./ Mult.	16	25	0	0	21	23	6	5	3	0	0	0	15	3	5	28	25
No. Lost	0	0	24	28	0	0	0	0	18	20	27	21	0	24	17	0	0

Twenty-eight legs with transdetermination induced by ectopic *wg* expression have been scored for specific pattern elements, such as sensilla groups and individual bristles, that have previously been localized on the foreleg disc fate map (Schubiger, 1968). Pattern elements have been scored as duplicated and/or multiplicated (Dup./Mult.), as lost, or as wild-type. Three of the 31 transdetermined legs could not be scored. V refers to ventral structures, D to dorsal structures. Pattern elements: BH$^-$, isolated bristle in hairy cuticle; St8, group of 8 sensilla trichodea; St3, row of 3 sensilla trichodea; St4, row of 4 sensilla trichodea; St5, row of 5 sensilla trichodea; Sc3, row of 3 sensilla campaniformia; GSt1, first group of sensilla trichodea; GSt2, second group of sensilla trichodea; Sc$^+$5, group of 5 sensilla campaniformia; St1, single sensillum trichodeum; EB, edge bristle; Sc$^-$8, group of 8 sensilla campaniformia; Sc11, group of 11 sensilla campaniformia; Sc1, single sensillum campaniforme; PA, preapical bristle; SC, sex comb; Claw, ungues of the claw organ. See Fig. 3B for positions of representative pattern elements on the fate map.

tures. Both leg discs and legs have duplicated proximal-distal axes (Figs 1C,D, 4B). Furthermore, Vg immunostaining in the leg discs and wing cuticle in adult legs are both found adjacent to the regions of dorsal loss (Figs 1C, 2D). Taken together, these observations indicate a correspondence among dorsal leg bifurcations, loss of dorsal leg structures, and transdetermination. One of these pattern deviations, dorsal leg bifurcations, may be induced by dorsally arising *wg*-expressing clones (Struhl and Basler, 1993; Campbell et al., 1993; Diaz-Benjumea and Cohen, 1994). Therefore, dorsal *wg*-expressing clones might also induce loss of dorsal leg structures and transdetermination.

A deficiency of dorsal leg cell clones reveals changes in dorsal leg cell fates

The results presented above suggest that *wg*-expressing clones can have four effects on dorsal leg cell fate. These clones may take on more ventral or more distal leg cell fates in dorsally positioned outgrowths, they might cause loss of dorsal leg structures, or they might transdetermine to wing. To assess more closely the fates of *wg*-expressing clones, we have analyzed clone frequencies and leg pattern deviations in several male foreleg segments. To analyze the fates of *y, wg*-expressing clones around the circumference of the leg, we have scored the eight regularly distributed longitudinal bristle rows of the femur (Hannah-Alava, 1958; Steiner, 1976; see Fig. 3B). To analyze the fates of *y, wg*-expressing clones along the proximal-distal axis of the leg, we have scored individual stereotypical bristles in many leg segments, as well as the claw (see Table 2). For both analyses, bristles have been scored for their presence and their phenotype (y^+ or y). This method of analysis reflects the ability of clones to arise at certain positions on the leg disc fate map and then to directly contribute to the bristles at those positions. As a control for the distribution of *y* clones, we have performed the same analyses in male forelegs with ectopic *sc* expression.

The results from these analyses are presented in Table 2. In control forelegs with *y, sc*-expressing clones, the femur's eight bristle rows and individual bristles in other leg segments are each marked with *y* with about the same frequency, indicating a random distribution of clones both around the circumference of the leg and along the proximal-distal axis of the leg. However, in forelegs with *y, wg*-expressing clones, the dorsal bristle rows of the femur are rarely marked with *y* relative to ventral bristle rows. Dorsal bristles in other leg segments (the edge bristle of the trochanter and the preapical bristle of the tibia) also are rarely marked with *y* (Table 2). This skewed distribution of *wg*-expressing clones supports the prediction that the fates of dorsal *wg*-expressing clones are altered. However, only 5% (11/202) of legs with *y, wg*-expressing clones have dorsally positioned outgrowths bifurcating from the femur, and no striking abundance of *y, wg*-expressing clones is found either in ventral femur rows or in distal ventral bristles (Table 2). Thus, although we cannot rule out that cell fate changes from dorsal leg to ventral or distal leg contribute to the loss of dorsal clones, it is unlikely that such changes alone account for the dramatic discrepancy in clone frequency. These analyses support the hypothesis that dorsal *wg*-expressing clones participate in other cell fate changes, such as transdetermination. Corresponding with the low frequency of dorsal *y, wg*-expressing clones is a high frequency of loss of dorsal leg bristles, including both dorsal femur rows and individual bristles along the proximal-distal axis (Table 2). An indication that transdetermination may contribute to this loss is that transdetermination is always observed adjacent to dorsal loss (see above).

Although transdetermination to wing in ectopic *wg*-expressing flies is never observed without loss of dorsal leg structures, dorsal loss can occur without transdetermination. Fourteen percent (28/202) of legs with *wg*-expressing clones show dorsal loss but no transdetermination. Thus dorsal loss may be a precondition for transdetermination. However, a localized effect of dorsal loss is likely not acting alone to cause transdetermination (see Discussion). Because gain of ventral structures also always accompanies transdetermination, multiple pattern regulation events may cooperate to induce transdetermination.

DISCUSSION

wingless and cell fate decisions

The results presented here lead to the conclusion that *wg*-expressing clones that arise in the dorsal region of the foreleg disc induce cell respecification events that cause loss of dorsal leg cell structures and transdetermination into wing. Much evidence points to *wg* having its most dramatic effects on leg

Table 2. Leg cuticle analyses correlate loss of dorsal *y*, *wg*-expressing clones with loss of dorsal leg structures

				Control (*n*=209 legs) *sc*-expressing clones		Experiment (*n*=202 legs) *wg*-expressing clones	
	Leg bristles			CLONE (%)	LOSS (%)	CLONE (%)	LOSS (%)
	Femur Row						
V		1		38	0	30	0
↓		2		36	0	17	0
		3		27	0	**9**	3
D		4		47	0	**1**	**10**
D		5		42	0	**3**	**7**
		6		36	0	**5**	1
↑		7		22	0	21	0
V		8		36	0	26	0
Prox.	Coxa	BH^-	V	31	0	16	1
	Troch.	EB	D	29	2	**1**	**12**
↓	Tibia	AV	V	20	0	9	0
		PA	D	23	0	**0.5**	**5**
↓	T1	CB	V	19	0	10	0
		AV	V	17	0	10	0
Dist.		Claw	D	14	0	23	0

Clones of ectopic *wg*- or *sc*-expressing cells are identified by the presence of *y* bristles within a femur row or at sites of individual bristles along the proximal-distal axis: the BH^- bristle of the coxa, the edge bristle (EB) of the trochanter, the tibia's large anterior-ventral bristle (AV) and preapical bristle (AP), the central bristle (CB) and most distal anterior-ventral bristle (AV) of the first tarsal segment (T1), and the claw. Ectopic *sc* expression is used as a control to demonstrate that the '*flp*-out' system can generate *y* clones distributed randomly around the fate map. Clone frequencies (CLONE columns) are given as the percent of legs which have ≥1 *y* bristle at those sites. Loss frequencies (LOSS columns) are given as the percent of legs that are missing an entire row of femur bristles or one of the individual bristles in other leg segments. Bold numbers indicate where striking losses of clones and leg structures occur. D and V refer to dorsal and ventral positions, respectively; prox.=proximal, dist.=distal leg segments. Fig. 3B shows where the BH^-, the EB, and the eight femur rows fate map on the foreleg disc.

pattern regulation when it is ectopically expressed in dorsal leg disc cells. Previous studies on the effects of ectopic *wg* expression have shown that proximal-distal axis bifurcations arise in dorsal leg disc cells (Struhl and Basler, 1993; Campbell et al., 1993; Diaz-Benjumea and Cohen, 1994). These dorsal outgrowths differentiate as ventrolateral leg structures that intercalate into the pattern of the endogenous leg. *wg*-expressing clones that contribute to these outgrowths then originally arise in dorsal positions, yet their fates become respecified to that of more ventral leg. We observe that the distribution of *wg*-expressing clones is skewed such that very few clones develop to mark dorsal leg bristles. While some dorsally arising *wg*-expressing clones develop to mark ventral and/or distal leg bristles in dorsal leg bifurcations, we can't account for the reduction of dorsal clone frequency based only on dorsal clones contributing to outgrowths. Thus either cell fate switches other than dorsal leg to ventral or distal leg are occurring, or many dorsally arising *wg*-expressing clones die. The possibility that *wg* acts via cell death to induce transdetermination remains to be addressed. However, many observations suggest that death of dorsal leg disc cells cannot by itself induce transdetermination. We always observe gain of ventral structures, as well as dorsal loss, accompanying transdetermination, suggesting that multiple pattern regulation events may cooperate to induce transdetermination. Random cell death generated either with a temperature-sensitive cell-lethal mutation or with X-irradiation does not induce transdetermination (M. Russell, personal communication; Postlethwait and Schneiderman, 1973). Furthermore, flies that have '*flp*-out' clones which ectopically express either of two other secreted signaling molecules, Hedgehog or Decapentaplegic, also have skewed clone distributions around the circumference of the leg and leg pattern deviations (although these clone distributions and pattern deviations differ from those observed with ectopic *wg* clones; Basler and Struhl, 1994; Diaz-Benjumea et al., 1994; L. Johnston, personal communication). However, these flies do not show leg-to-wing transdetermination (L. Johnston, personal communication). Thus, *wg* is likely acting specifically to induce certain cell fate changes, including dorsal leg to ventral wing.

One explanation for both the loss of dorsal *wg*-expressing clones and the leg pattern deviations that we observe (gain of ventral leg structures and loss of dorsal leg structures accompanying transdetermination to wing) is that a dorsal *wg*-expressing clone might induce extensive proliferation, a prerequisite for transdetermination, in neighboring dorsal leg disc cells. These dividing wild-type (y^+) cells could out-compete the *y*, *wg*-expressing clone, which would effectively cause dorsal clones to be 'lost.' The proliferating y^+ cells could take on more ventral leg fates, leading to the observed gain of ventral leg structures and loss of dorsal leg structures.

Wilder and Perrimon (1995) have recently studied the effects of targeted ectopic expression of *wg*. They find that flies that ectopically express *wg* either ubiquitously in leg discs or along the leg disc A/P boundary have a significant amount of loss of dorsal leg structures. However, these flies do not exhibit leg-to-wing transdetermination (Wilder and Perrimon, 1995; L. M. and G. S., unpublished observations). These results appear to contradict the conclusion that ectopic *wg* expression in dorsal leg disc cells can induce transdetermination. However, there are several fundamental differences between these targeted expression experiments and the experiments described in this paper. The '*flp*-out' clone experiments express a wild-type Wg protein. The targeted expression studies use a temperature-sensitive Wg protein, which may not have the complete range of wild-type function. Indeed, the gain of ventral leg structures in flies with targeted expression of the Wg temperature-sensitive protein is not as extensive as that observed in flies with ectopic *wg*-expressing clones (Wilder and Perrimon, 1995; L. M. and G. S., unpublished observations). The targeted expression studies drive more widespread ectopic expression of *wg* than is induced in the '*flp*-out' *wg* clone studies. More extensive ectopic *wg* expression may induce pattern regulation differently than ectopic *wg* clones.

A comparison of transdetermination experiments

The foreleg-to-wing transdetermination event induced by ectopic *wg* expression in many respects resembles the foreleg-to-wing transdetermination that occurs readily in cultured disc fragments. Here we compare the results of these two methods that elicit transdetermination. The similarities between disc fragmentation experiments and ectopic *wg* expression experiments underscore a fundamental role for *wg* in transdetermination.

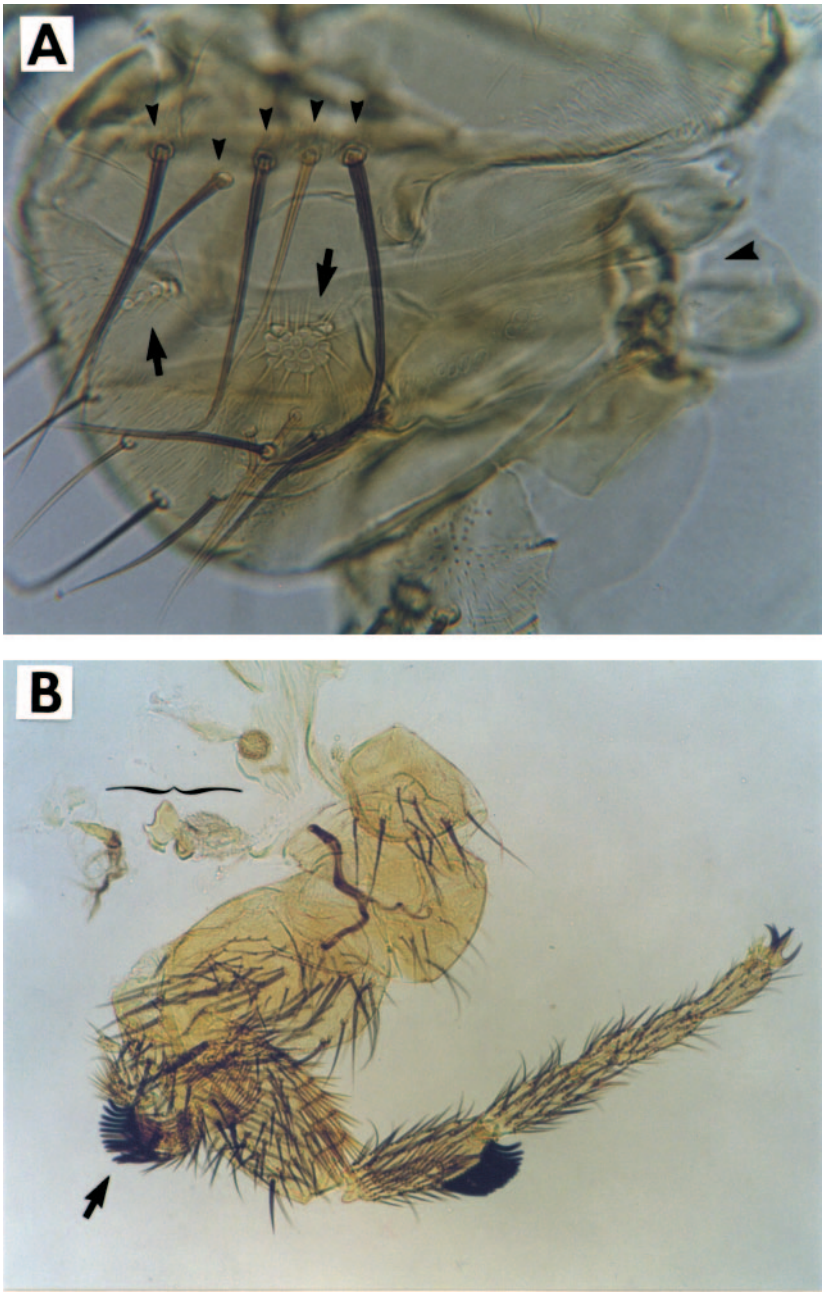

Fig. 4. Gain of ventral leg structures and loss of dorsal leg structures in transdetermined legs. (A) High magnification view of the proximal coxa and trochanter segments from a male foreleg with *y*, *wg*-expressing clones and transdetermination. The ventral BH^- bristle of the coxa is multiplied from 1 bristle to 5 (small arrowheads). The trochanter's ventral St5 group of sensilla has duplicated to two groups (arrows), and one group has multiplied from 5 to 17 sensilla. The circumference of the trochanter segment is open on the dorsal side (large arrowhead). The dorsal edge bristle normally found at this position is absent. The transdetermined wing structures are found in more distal segments of this leg. (B) Male foreleg with *y*, *wg*-expressing clones and transdetermination. Wing hinge structures are found adjacent to the dorsal side of the trochanter segment (bracket). This leg bifurcates from the dorsal side of the tibia. The outgrowth consists of ventrolateral leg structures (arrow points to extra sex comb) and has a *y*, *wg*-expressing clone (note *y* sex comb bristles). Dorsal loss occurs in this leg proximal, but not distal, to the outgrowth. Magnification is ×400 in A and ×100 in B.

Similar leg pattern deviations are produced

In foreleg disc fragmentation and culture experiments, Schubiger (1971) found that regeneration and multiplication of leg pattern elements, such as bristles and sensilla, always accompanied transdetermination. In leg disc fragments that transdetermine, ventral leg structures are preferentially regenerated or multiplied, whereas dorsal leg structures are less frequently recovered (Schubiger, 1971). Gain of ventral leg structures and loss of dorsal leg structures are also observed after inducing ectopic *wg* expression. Schubiger (1971) interpreted multiplication events as a sign of excess proliferation, which might then promote transdetermination. The results presented here suggest that ectopic *wg* expression mimics the fragmentation experiments by inducing the formation of a proliferating blastema in the absence of a wound. *wg* may play a role in promoting this proliferation (Skaer and Martinez Arias, 1992; Kaphingst and Kunes, 1994). Consistent with this hypothesis, Brook et al. (1994) found, in cultured leg disc fragments, that *wg* expression expands from its normal ventral domain of expression to the site of the blastema.

Although proliferation is required for transdetermination, pattern regulation events must accompany the proliferation. In both disc fragmentation and ectopic *wg* expression experiments, gain of ventral and loss of dorsal leg structures may be prerequisites for transdetermination because, although the former events can occur in the absence of transdetermination, transdetermination never appears without them. Furthermore, we find a correspondence between transdetermination, dorsal loss, and dorsally positioned leg outgrowths. All transdetermined legs lose some dorsal leg structures, although the loss

is less extensive in legs with outgrowths. Dorsally arising *wg*-expressing clones likely induce these events.

Similar wing structures are produced

The transdetermined wing structures that we observe in legs with ectopic *wg* expression all fate map to the presumptive ventral wing hinge region of the wing disc. These wing structures are the same as the initial transdetermined structures that arise from cultured leg disc fragments; longer culture times allow other wing structures, such as the blade and the margin, to also be produced (Schubiger, 1968). Thus, ectopic *wg* expression acts in lieu of disc fragmentation to initiate transdetermination in developing leg discs. This in situ transdetermination probably does not allow enough 'culture' time to generate more extensive wing structures.

wg is normally expressed in the presumptive ventral region of the wing disc at the second and early third instar stages (Couso et al., 1993; Williams et al., 1993) and is required for the formation of ventral wing structures (Morata and Lawrence, 1977; Williams et al., 1993). Thus, *wg* also likely plays an important role in producing the specific ventral wing hinge structures in legs that transdetermine. Indeed, we always observe *y*, *wg*-expressing clones in leg bristles adjacent to the transdetermined wing structures.

Transdetermining cells localize to the same region

Strub (1977), after compiling data from foreleg disc fragmentation experiments, localized transdetermining cells to the proximal-dorsal region of the foreleg disc. We provide a molecular confirmation for the site of foreleg disc transdetermination by showing that Vg immunostaining occurs in the same region predicted by Strub. Foreleg disc fragments that 'expose' these dorsal cells at a wound surface are the only fragments that give rise to transdetermination (Strub, 1977). Transdetermining leg disc fragments also require a ventral cut edge, possibly because proliferating blastemas form most readily at ventral cut edges. Thus the ectopic *wg* expression experiments may mimic the fragmentation experiments by juxtaposing 'ventral' *wg*-expressing cells with dorsal leg disc cells.

How does *wingless* induce transdetermination?

Dorsal leg disc cells express high levels of *decapentaplegic* (*dpp*), a *Drosophila* TGF-β homologue (Masucci et al., 1990; reviewed by Gelbart, 1989). Interactions between *wg* and *dpp* have been shown to affect patterning in the *Drosophila* brain (Kaphingst and Kunes, 1994), midgut (Mathies et al., 1994) and leg (Held et al., 1994). Intersections between *wg*- and *dpp*-expressing cells are thought to specify the thoracic imaginal primordia (Cohen et al., 1993) and the distal end of the proximal-distal axis in developing leg discs (Campbell et al., 1993). An interaction between *dpp* and ectopic *wg* in dorsal leg disc cells has been implicated in inducing supernumerary leg outgrowths (Campbell et al., 1993). We speculate that an interaction between a clone of ectopic *wg*-expressing cells and *dpp*-expressing cells in the dorsal region of the foreleg disc also induces transdetermination. It may be that high levels of *wg* activity in dorsal leg cells induces transdetermination and loss of dorsal leg structures in addition to leg bifurcations, whereas lower ectopic levels of *wg* may induce only leg bifurcations. Although *wg* and *dpp* expression normally coincide in the ventral region of leg discs, high ventral levels of *wg* are thought to repress *dpp* activity (Held et al., 1994). Indeed, the phenotype of transdetermined legs resembles that of reduction-of-function *dpp* mutants (gain of ventral leg structures and loss of dorsal leg structures; Held et al., 1994), suggesting a repression of *dpp* activity. Also consistent with a *wg-dpp* interaction is that the transdetermined wing structures fate map to a region of the wing disc that expresses both molecules at high levels, particularly during mid-larval development (Masucci et al., 1990; Couso et al., 1993; Williams et al., 1993), when the ectopic expression of *wg* is induced in the experiments presented here. Thus, relative levels of these signaling molecules at particular stages of development may be responsible for affecting cell fate decisions, and aberrant expression of *wg* during a critical period may cause dorsal leg cells to transdetermine to wing cells.

Now that we have identified *wg* as a stimulus for transdetermination, one approach to dissect further the process of transdetermination will be to determine the cell signaling pathways that *wg* acts through to induce the leg-to-wing switch. *wg* regulates homeotic gene expression in the midgut (Thüringer and Bienz, 1993; Thüringer et al., 1993) and may induce a change in homeotic gene expression during imaginal disc cell transdetermination. If so, *wg* may be able to affect a variety of transdetermination events in other disc types. Because limb patterning in insects and vertebrates appears to proceed using similar signaling molecules (Riddle et al., 1993; Basler and Struhl, 1994), an understanding of transdetermination in *Drosophila* imaginal discs may provide insight into transdetermination phenomena that have been observed in other insect appendages and amphibian limbs.

We thank Gary Struhl for fly stocks, Sean Carroll for the Vg antibody, Jayne Baker for help with computer imaging, Mike Russell and Laura Johnston for permission to cite unpublished results, and Lynn Riddiford, Margrit Schubiger and members of the Schubiger lab, especially Laura Johnston, for comments on the manuscript. L. M. is supported by an NIH predoctoral training grant and an ARCS Fellowship. This work was supported by NIH grant GM 33656 to G. S.

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Accepted 10 February 1995)